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Enlarged hind wings of the Neotropical butterfly Pierella helvina (Nymphalidae) enhance gliding flight performance in ground effect.

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Abstract

Flight is a vital component of butterfly natural history, and flight-associated morphology is thought to be under strong selection for the performance of critical behaviors such as patrolling, courtship and oviposition. However, while different behaviors require different proportions of flapping versus gliding flight, few studies actually quantify butterfly flight behavior. Moreover, as butterfly flight is anteromotoric, no prior study has measured the role of hind wing allometry in flight. Using high-speed videography, this study compares the flight of two species of Haeterini (Nymphalidae) that regularly employ gliding flight. We also employ stereo videography and experimental hind wing area reduction to measure the effects of hind wing allometry on flight. Results suggest that although the forewings are reliable predictors of flight in these two species, relative hind wing area can significantly affect gliding flight performance, and should be considered as a factor in future investigations on flight-associated morphology in butterflies.

Keywords: Hind wings; gliding butterfly flight; Haeterini; *Pierella helvina*; *Cithaerias pireta*; stereo videography

Introduction

Flight is intimately linked to butterfly behavior and natural history, and selection on the performance of critical behaviors such as predator avoidance, mate-finding, courtship and oviposition has led to a broad diversification of butterfly wing and body morphology. When utilizing flapping flight, thoracic muscle mass, wing loading, and forewing centroid position have all been shown to affect flight speeds (Bartholomew & Casey 1978, Dudley 2000). In contrast, gliding flight performance is enhanced by a high forewing aspect ratio that increases lift and reduces drag, thus promoting energy efficiency (Dudley 2000; see also Marden 1987, Marden and Chai 1991, DeVries et al. 2010).

As the forewings are the first point of contact between the wings and incident air flow, pressure gradients tend to be highest around the leading edge of the forewings (Ancel et al. 2016), and it is not surprising that forewing morphology is considered the prime determinant of flight performance in butterflies (Dudley 2000, Berwaerts et al. 2006, Jantzen and Eisner 2008, Li et al. 2016, Le Roy et al. 2019). Among rainforest butterflies, many that fly in the canopy tend to exhibit extensive gliding behavior and also high forewing aspect ratio (DeVries et al. 2010). In general, male butterflies have a higher forewing aspect ratio than conspecific females, especially in species where males glide to patrol mating territories (Betts and Wootton 1988, Berwaerts et al. 2002, DeVries et al. 2010, Cespedes et al. 2014). Selection is thus expected to influence the shape and aerodynamic properties of the forewings in order to maximize performance during critical flight behaviors (DeVries et al. 2010, Shi et al. 2015, Chazot et al. 2016).

While the relationship between forewing morphology and flight is well established, comparatively little is known about the contribution of hind wings to butterfly flight (Le Roy et al. 2019). Using a moth and a butterfly species, Jantzen and Eisner (2008) showed experimentally that while hind wing removal caused a decrease in acceleration and maneuverability, both species were capable of flight using the forewings only. The butterfly in that study, *Pieris rapae* (Linnaeus, 1758) (Pieridae), utilizes flapping flight almost exclusively and its wide forewings presumably function to enhance flapping flight efficiency (Dudley 2000,

Ha et al. 2013). To our knowledge, no study has evaluated the aerodynamic role of hind wings in a butterfly that regularly employs gliding flight.

Various morphological parameters are known to play a role in butterfly gliding flight. Since the induced drag on a wing scales with its cord length (Dudley 2000), selection for gliding efficiency should minimize cord by producing elongate forewings with high aspect ratio (see Le Roy et al. 2019 for a review). As forewings and hind wings operate in concert, the size and shape of the hind wings can influence induced drag as they contribute to the cord length of the wing pair (Dudley 2000, Ancel et al. 2016). Although the lift generated by the wings is proportional to their area (Dudley 2000), an enlarged hind wing would result in increased induced drag — thus constraining the contribution of the hind wing to gliding flight. This constraint is relaxed by the aerodynamic phenomenon of ground effect that occurs when a wing experiences a reduction in induced drag during gliding due to its close proximity to a fixed surface (Rozhdestvensky 2000, Cui and Zhang 2010, Rahimuddin et al. 2014). The strength of ground effect is inversely proportional to flight altitude, and wings of animals that regularly glide close to a surface should be optimized to take advantage of this phenomenon (Withers and Timko 1977, Hainsworth 1988, Rayner 1991). Butterflies that glide in ground effect therefore present a unique opportunity to study hind wing aerodynamics.

Most species in the Neotropical butterfly tribe Haeterini (Nymphalidae, Satyrinae) employ a significant amount of gliding flight while performing routine behaviors like finding food, patrolling territories or searching for oviposition sites (Weymer 1910, Zikan 1942, Masters 1970, DeVries 1987). Observations and mark-recapture studies suggest that males use patrolling flight to move extensively within their home ranges presumably as part of mate seeking behavior, whereas females move through the forest understory searching for larval host plants without showing home range behavior (DeVries and Alexander unpublished data, see also Murillo-Hiller 2009). Finally, a comparative study in Costa Rica employing fruit-baited traps positioned at 15 cm and 1 m above the ground found that *Pierella helvina* (Hewitson, 1860) was sampled only in lower traps while *Cithaerias pireta* (Stoll, 1780) were captured in both (Alexander and DeVries 2012). These field observations are consistent with the suggestion that there has been wing shape evolution in some Haeterini to enhance aerodynamic efficiency for gliding in ground effect (Cespedes et al. 2014). The apparent importance of gliding flight in these two species, combined with notable differences in both forewing and hind wing

morphology between species made *P. helvina* and *C. pireta* the ideal system in which to investigate the role of wing morphology in gliding butterfly flight.

While both *P. helvina* and *C. pireta* frequently use gliding flight in the forest understory, *P. helvina* appears to have larger hind wings relative to the forewings than does *C. pireta* (Fig. 1, and http://butterfliesofamerica.com/t/Haeterini_a.htm, last accessed 14 December 2018). Observations of flight behavior and wing morphology thus led us to hypothesize that the enlarged hind wings of *P. helvina* could function to enhance gliding flight in ground effect. To test this hypothesis, we compared the wing morphology and flight performance of *P. helvina* to *C. pireta*. We measured forewing and hind wing areas and forewing aspect ratio to assess potential differences between sexes and species. We then used high-speed videography to address the following questions relevant to flight performance: (1) Does gliding flight in *P. helvina* and *C. pireta* conform to predictions based on forewing aspect ratio alone? (2) Does the large hind wing area affect the gliding flight of *P. helvina*? (3) Does hind wing area affect the flight speed or maneuverability of *P. helvina*?

Methods

Study site and sampling

This investigation was conducted at the Tirimbina Biological Reserve, Heredia Province, Costa Rica (10°29'50.3''S; 76°22'28.9''W) in December 2016. The Tirimbina 345 hectare reserve is composed of approximately 85% primary lowland rain forest, and has an elevation range of 180–220 m. See DeVries et al. (2011) for a more detailed description.

Individuals of *P. helvina* and *C. pireta* were captured in the field with hand nets between 0700 – 1100 h, placed in glassine envelopes, and subsequently transferred to 500 ml transparent plastic containers kept at ambient temperature in the lab. To maintain a good physical condition, all individuals were allowed to feed *ad libitum* on overripe fruit. Butterflies captured on a given morning were stored for no more than 3 h before experimental flights in the lab. The ambient temperature indoor flight arena consisted of an open room (ca. 4 x 5 m in area) with ambient light from a standard size screened door.

Wing measurements and manipulations

Captured specimens were photographed next to a metric scale, and the images were processed using Adobe Photoshop® to yield a cutout of each wing to measure length and area (Combes and Daniel 2003). We standardized wing length measurements by fitting the smallest possible circle around each cutout in Adobe Photoshop®, and the diameter of this circle corresponded to wing length. The left wing pair was used to measure length and area. To calculate aspect ratio we used the equation AR = (span^2)/(total FW area) where span refers to twice the length of the forewing. The combined areas of one forewing and one hind wing were doubled to estimate total wing area. All measurements were performed with the NIH software ImageJ (https://imagej.nih.gov/ij/, last accessed January 2017).

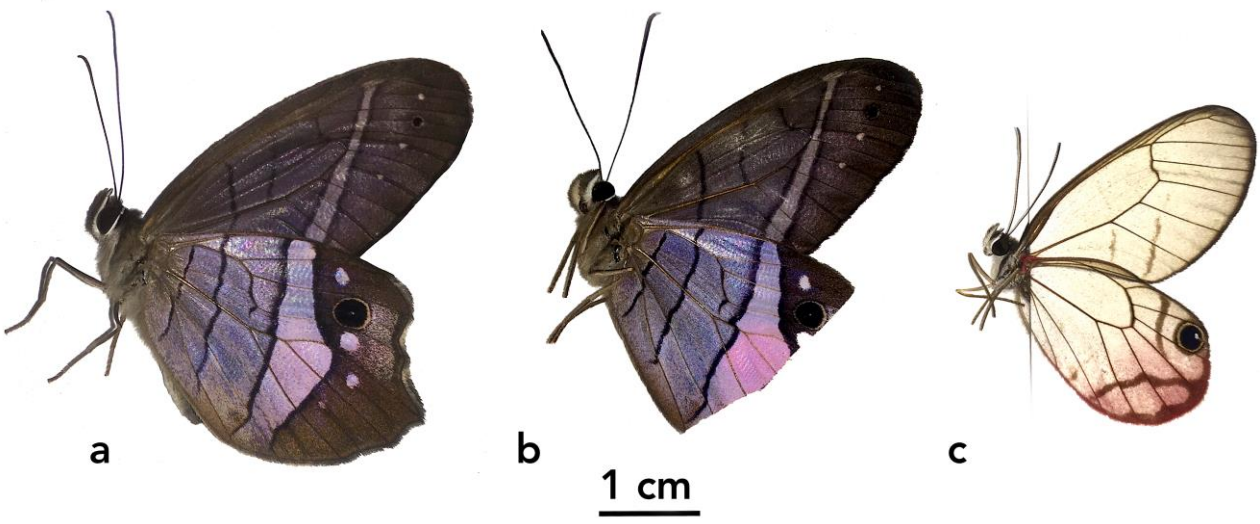

Fig. 1. Male *Pierella helvina* with intact (**a**) and reduced hind wings (**b**). Male *Cithaerias pireta (***c**).

To explore the functional role of the enlarged hind wing of *P. helvina* (Fig. 1a), we experimentally trimmed hind wings of male and female specimens (Fig. 1b) to approximate the relative hind wing area of *C. pireta* (Fig 1c). Two straight cuts were made across the paired hind wings of each individual to approximate a 10% reduction in hind wing contribution to total wing area. Only butterflies with undamaged hind wings were used for experimental hind wing size reduction, yielding a total of fourteen individuals (7 males, 7 females) from which flight data were collected. After cutting, no hemolymph was visible from the wings and individuals did not

show signs of behavioral trauma. To measure the percent reduction in hind wing area we compared photographs of individuals before and after the wings had been cut.

Videography

We used iPhones SE®, 240 fps (Apple Inc.) to record high-speed videos of live butterfly flight in the field, and in the laboratory. Due to limitations in light levels in the rainforest understory, the methods for filming in the field differed from those used in the laboratory.

To record the natural flight behavior of *P. helvina* and *C. pireta*, butterflies were video-taped in forest light gaps, and as these individuals seem to be patrolling, we assumed they were males. As they were not captured and sexed, their flight data were pooled by species. While flapping and gliding flight was discernable in these videos, limited light precluded accurate kinematic analysis, estimates of flight path, or flight speeds for such individuals. A flight in the field was deemed suitable for frame-by-frame analysis if at least five consecutive wing-beat cycles were captured by a single camera. For each species we recovered sixteen individual, usable flights.

To assess flight performance in the lab, two high-speed video cameras were set up in stereo to create an overlapping field of view (i.e., the flight arena). Video-capture in stereo allowed us to use the angle of a flying butterfly from each of the cameras to triangulate its three-dimensional position within the flight arena (Almbro and Kullberg 2007, Chakravarthy et al. 2009**,** deMargerie 2015; Supplementary Figure S1). These kinematic data, plus temporal data derived from the precise frame rate of each camera were used to estimate individual flight path, speed, glide duration, wing-beat frequency, wing-beats per total flight time, and the ratio of gliding to total flight time.

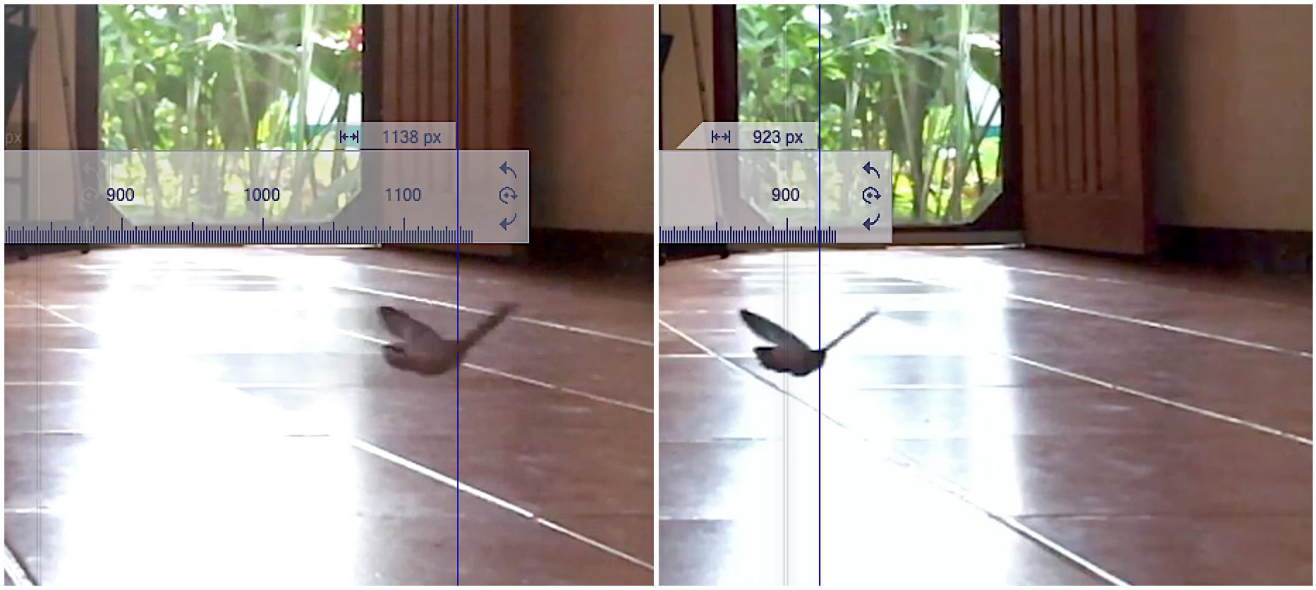

Fig. 2. Still images from two concurrent videos of a male *P. helvina* gliding in the lab depicting the measurement of distance (in pixels) of the butterfly from the left-of-frame using ONDE RULERS. See Supplementary Figure S1 for further information.

Experimental Flight Protocol

At the start of each experimental flight, we used a light pulse from a flashlight to synchronize both cameras, and then an individual butterfly was gently placed on the floor approximately 1 m from the cameras in the center of the flight arena. Once placed, individuals either took flight immediately or were gently prodded on a hind leg to induce flight. We assume that these flights constituted reasonable approximations of the maximum flight capabilities when startled (escape-flight; see Almbro and Kullberg 2007), and that conditions affecting flight behavior were approximately constant for all experimental individuals.

After an individual with intact wings had been video-taped for two consecutive flights, the hind wings were surgically reduced, and it was immediately flown and video-taped twice more. These butterflies were sacrificed and stored in glassine envelopes as voucher specimens.

Video Analysis and Data Extraction

When the two videos of each flight were synced, they remained consistent on a frame-by-frame basis. Consistency at a frame rate of 240 fps, yields a high degree of precision in the measurement of flight time by frame-number (Dudley 1990, Brodsky 1991, Jantzen and Eisner

2008). Since flight speed, wing-beat frequency, and glide duration all depend directly on the measurement of time, such temporal precision is critical to our analyses.

Video-taping each flight in stereo enabled the triangulation of positional (x,y,z) data when butterflies were simultaneously visible to both cameras. Analysis of positional data of all butterflies was limited to the first five wing-beats of each flight. To extrapolate positional data from raw digital videos, it was first necessary to construct a standard curve relating the distance of a butterfly from left-of-frame to the angle of that butterfly from each camera. This was measured in pixels using ONDE RULERS for Mac (Fig. 2). These two angles were then used to triangulate the (x,y) position of a butterfly at the beginning (pronation), middle (supination), and end (pronation) of each wing-beat cycle. Height (z) was measured in a similar fashion. See Supplementary Table S1 and Figure S1 for detailed descriptions of these positional calculations. To test the accuracy of our stereo camera rig, a tape measure was set in the flight arena parallel to, and 1.5 m from, the camera plane, and the (x,y,z) position of each marking on the tape was measured using ONDE RULERS as described above. Triangulation of over 50 points of known position produced an average error of 3.56 mm and a maximum error of 8.32 mm, which was considered sufficiently accurate for kinematic measurements (Jantzen and Eisner 2008, Chakravarthy et al. 2009).

Flight Data

To compare the flight behaviors of *P. helvina* and *C. pireta* in the field and in the lab, we used temporal data derived from the precise frame rate of each camera. To quantify gliding flight, we measured glide durations and the proportion of gliding to total flight time for each individual (Dudley 1990). To quantify flapping flight, we measured both wing-beat frequency and the number of wing-beats per total flight time for each individual.

To compare the kinematic flight performance of *P. helvina* before and after experimental hind wing reduction, we used (x,y,z) positional flight data to measure both flight speeds and flight path for each individual. Flight paths were used to calculate sinuosity, a measure of curvilinearity, as a proxy for maneuverability (Almbro and Kullberg 2007).

Statistical analyses

We used one-tailed t-tests to assess differences in wing morphology between species and sexes, as these data fit a normal distribution. Mann-Whitney U tests were used to compare proportions of gliding per total flight time and wing-beats per total flight time because these proportions were homoscedastic, but not normally distributed. We used Kruskal-Wallis tests to compare samples of wing-beat frequencies and glide durations because these variables were not normally distributed and heteroscedastic. All analyses were performed in the statistical suite JASP (Version 0.9.1) for Mac. See Supplementary Table S2 for results of Shapiro-Wilk and Levene's tests for normality and homoscedasticity, and a compilation of all statistical tests performed in this study.

Results

Table 1. Comparisons of relative area of the hind wings (HW) and forewing aspect ratio (FW AR) between *P. helvina* and *C. pireta*.

	HW/Total Wing Area (%)	FW AR
Male *P. helvina* (n=7)	56 ± 1.2	5.77 ± 0.17
Male *C. pireta* (n=7)	46 ± 0.3	5.34 ± 0.13
P-value	**3.83E-10**	**1.62E-04**
Female *P. helvina* (n=7)	55 ± 1.2	5.45 ± 0.18
Female *C. pireta* (n=7)	47 ± 0.4	5.35 ± 0.09
P-value	**1.84E-06**	0.159
Male vs. Female *P. helvina*, P-value	**0.026**	**0.002**
Male vs. Female *C. pireta*, P-value	0.056	0.484

Values are sample means and standard deviations. P-values correspond to one-tailed t-tests between samples. Sample sizes (n) are in parentheses.

Wing morphometrics

In both sexes, the hind wings of *P. helvina* comprised a greater portion of the total wing area than those of *C. pireta* (Table 1, Fig. 3). Male *P. helvina* had larger hind wings than conspecific females, but in *C. pireta* the sexes were the same (Table 1). Experimental hind wing reduction in *P. helvina* (Fig. 1b) resulted in a similar relative hind wing area as possessed by *C. pireta* (Fig. 1c, 3).

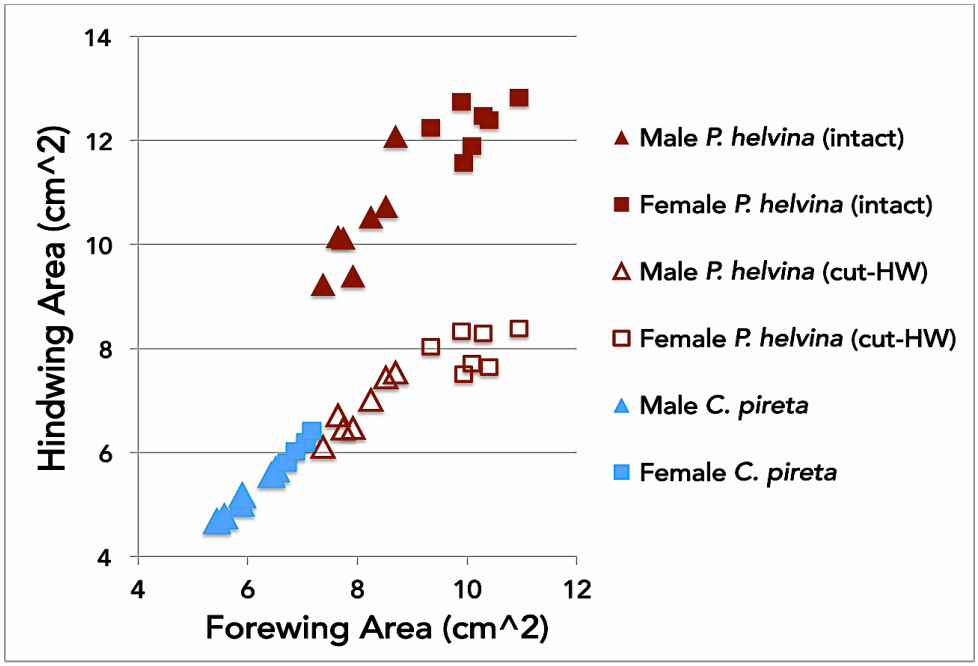

Fig. 3. Allometric intercepts for individual *Pierella helvina* with intact and cut hind wings, and *Cithaerias pireta* with intact wings.

Male *P. helvina* had significantly higher forewing aspect ratio than male *C. pireta*, which was not the case for females of these species (Table 1). We also found that male *P. helvina* had significantly higher forewing aspect ratio than conspecific females, but *C. pireta* showed no difference between the sexes (Table 1).

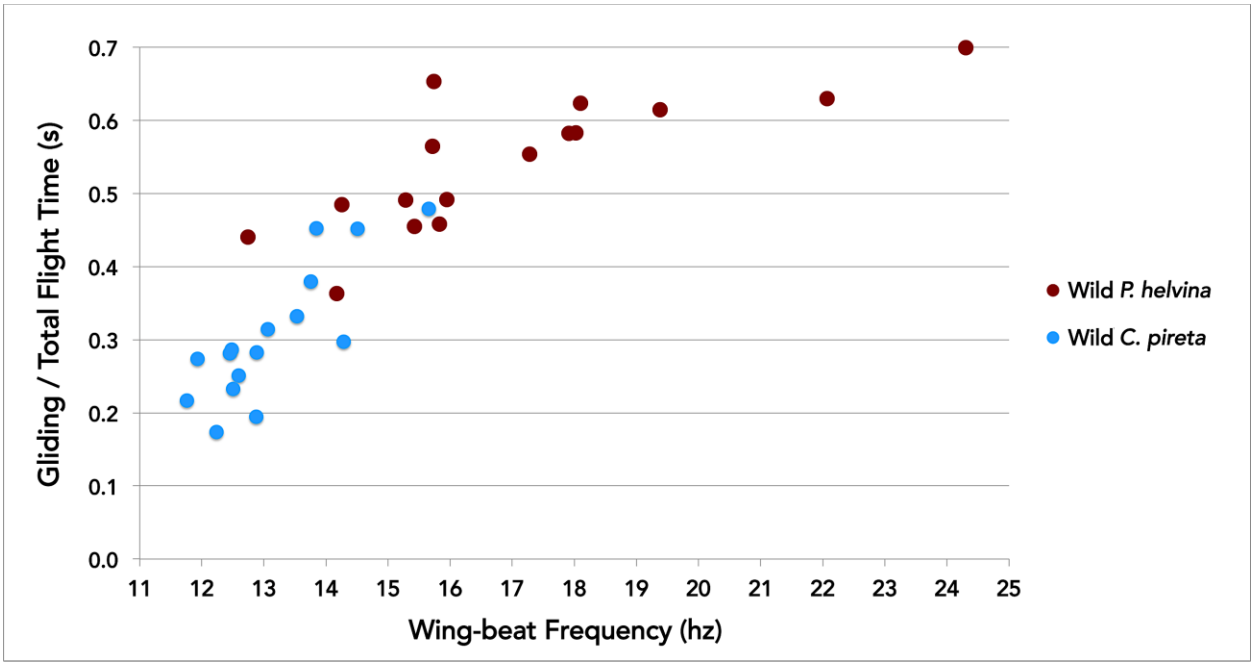

Fig. 4. Gliding flight for undisturbed *P. helvina* and *C. pireta* individuals in the field. Each point represents data accumulated over the total observed flight time of a single individual in free flight.

Flight behavior in the field

We found that *P. helvina* exhibited greater glide durations and proportions of gliding to total flight time in the field than *C. pireta* (Table 2). Our results showed that *P. helvina* exhibited a narrower range of variation in gliding time than *C. pireta* (Table 2). Although *P. helvina* had greater wing-beat frequencies than *C. pireta*, they employed fewer wing-beats per total flight time—likely because they spent more time gliding (Table 2). The proportion of gliding was positively correlated with wing-beat frequency (Fig. 4) and negatively correlated with wing-beats per total flight time in both species (Fig. 5a). We assumed that individuals video-taped in the field were patrolling males.

Table 2. Comparison of flight performance between *P. helvina* (intact wings) and *C. pireta* in the field and laboratory.

Sample (n)	Glide Duration (ms)*	Gliding / Total Flight Time	Wing-beat Frequency (hz)	Wing-beats / Total Flight Time **
FIELD				
P. helvina (n=16)	96 (17–396)	0.56 (0.36–0.70)	15.9 (12.7–24.3)	7.5 (5.45–9.02)
C. pireta (n=16)	63 (21–179)	0.28 (0.17–0.48)	12.9 (11.8–15.7)	9.0 (7.58–10.37)
P-value	**1.9E-09**	**3.2E-07**	**4.03E-06**	**1.5E-05**
LABORATORY				
Male *P. helvina* (n=7)	67 (8–166)	0.35 (0.11–0.38)	13.0 (12.3 – 14.6)	8.37 (7.93–11.94)
Male *C. pireta* (n=6)	50 (21–121)	0.18 (0.11–0.25)	11.1 (10.1–13.1)	8.74 (8.27–11.71)
P-value	**0.0340**	0.073	**0.022**	0.731
Female *P. helvina* (n=7)	29 (4–171)	0.16 (0.04–0.25)	11.0 (10.1–13.1)	10.45 (8.06–11.00)
Female *C. pireta* (n=3)	42 (17–88)	0.14 (0.11–0.19)	11.5 (11.0–14.2)	9.53 (9.36–12.61)
P-value	0.332	0.833	0.383	0.667

Sample sizes (n) are in parentheses. Values are sample medians followed by ranges in parentheses. Kruskal-Wallis tests were used for comparisons of glide duration and wing-beat frequency between groups, as these data failed both tests of normality (Shapiro-Wilks) and homoscedasticity (Levene's Test). Mann-Whitney U tests between sample distributions were used for all other comparisons.
* Individual gliding events were pooled for analysis, where glide duration for a given sample reflects all gliding events of all flights for all individuals in that sample. For specific sample sizes, see Supplementary Table S2.
** Wing-beats / Total Flight Time is the amount of flapping per second of a recorded flight as a measure of flight energy expenditure.

Effects of hind wing reduction on *P. helvina* flight performance

Experimental reduction of hind wing area had an effect on *P. helvina* flight performance. We found that after hind wing area reduction individuals of both sexes showed an overall decline in gliding ability (Fig. 5b), but only males had a significant decrease in glide duration over the total flight time (Table 3). While only females exhibited a significant increase in wing-beat frequency, both sexes had a greater number of wing-beats per total flight time, but this difference was not significant (Table 3). Analysis of lab-flown *P. helvina* indicated that all butterflies reached a stable forward velocity within two wing-beats from takeoff (Fig. 6). This led us to

partition forward velocity into "escape velocity" and "cruising velocity", where the former represents the total distance travelled after the first wing-beat divided by the time interval of that wing-beat ~~(escape velocity)~~ and the latter represents the stable rate of forward motion during the third to fifth wing-beats ~~(cruising velocity)~~. We found that escape velocity decreased in both sexes after hind wing area reduction, but females were more strongly affected than males (Table 3). In contrast, cruising velocity was not affected by hind wing reduction in either sex. Lastly, as most flight paths were not long or varied enough, we could not detect differences in maneuverability (sinuosity). In sum, experimental hind wing reduction in male *P. helvina* significantly diminished gliding flight performance, but in females it produced both an increase in wing-beat frequency and a decrease in escape velocity (Table 3, Figs. 5b and 6).

Table 3. Comparisons of *P. helvina* flight performance with intact (Full-HW) and reduced hind wings (Cut-HW).

	Males (n=7)			Females (n=7)		
	Full-HW	Cut-HW	P-value	Full-HW	Cut-HW	P-
Glide Duration (ms)*	67 (8–166)	48 (4-100)	**3.85E-04**	29 (4–171)	35 (0–163)	0.269
Gliding / Total Flight Time	0.35 (0.11–0.38)	0.13 (0.04–0.24)	**0.026**	0.16 (0.04–0.25)	0.04 (0.00–0.15)	0.097
Wing-beats / Total Flight Time**	8.37 (7.93-11.94)	11.58 (8.91–13.85)	0.073	10.45 (8.06–11.00)	12.38 (10.28–13.94)	0.053
Wing-beat Frequency (hz)	13.0 (12.3 – 14.6)	12.2 (10.3–14.6)	0.406	11.0 (10.1–13.1)	12.7 (11.9–14.2)	**0.025**
Escape Velocity (m/s)	0.40 (0.28–0.59)	0.39 (0.08–0.66)	0.437	0.37 (0.15–0.58)	0.30 (0.22–0.41)	**0.037**
Cruising Velocity (m/s)	1.1 (0.68–1.91)	1.08 (0.69–1.76)	0.503	1.13 (0.83–2.25)	1.38 (0.91–1.84)	0.413
Sinuosity	1.11 (1.08–1.55)	1.12 (1.02–1.75)	0.936	1.18 (1.05–1.66)	1.13 (1.04–1.69)	0.347

Sample sizes (n) are in parentheses. Values are sample medians followed by ranges in parentheses. Kruskal-Wallis tests were used for comparisons of glide duration and wing-beat frequency between groups, as these data failed both tests of normality (Shapiro-Wilks) and homoscedasticity (Levene's Test). Mann-Whitney U tests between sample distributions were used for all other comparisons.
* Individual gliding events were pooled for analysis, where glide duration for a given sample reflects all gliding events of all flights for all individuals in that sample. Individuals that did not glide were recorded as a single glide duration of 0 ms. For specific sample sizes, see Supplementary Table S2.
** Wing-beats / Total Flight Time is the amount of flapping per second of a recorded flight as a measure of flight energy expenditure.

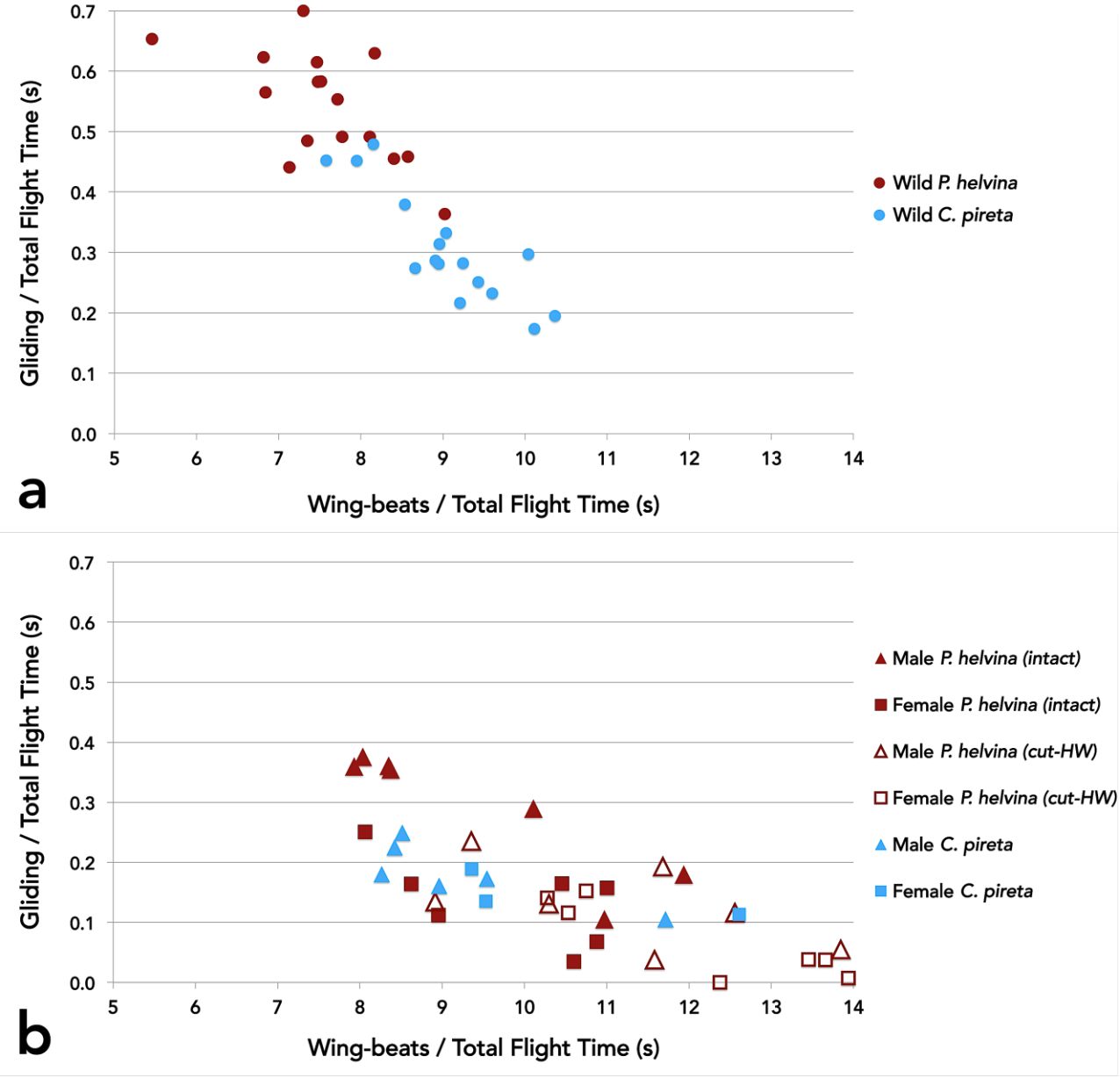

Fig. 5. Tradeoff between gliding and flapping flight of *Pierella helvina* and *Cithaerias pireta*. Each point represents data accumulated over the total flight time observed for an individual either in the field (**a)** or in the lab (**b)**. Individuals filmed in the field were not sexed but were likely males. Males and females of both species flown in the lab are represented by triangles and squares.

Comparisons between *P. helvina* and *C. pireta*

A comparison between undisturbed flight in the field and experimental flights of butterflies with intact wings allowed us to assess changes in gliding flight due laboratory conditions. Although natural and lab flights did not use the same individuals, Fig. 5 suggests that butterflies of both species generally spent more time gliding over total flight time in the field than in the lab. This can be interpreted as an effect of being flown in an enclosure and responding to a startle stimulus (see Methods).

All butterflies flown in the lab took off from the ground and ascended towards a source of natural light; i.e., during bouts of gliding flight, they were not taking advantage of ground effect. In spite of this, we were able to detect differences in flight performance between species and sexes. Male *P. helvina* with intact wings showed significantly longer glide durations than male *C. pireta*, but the range of individual variation was broader in *P. helvina* (Table 2). Although the proportion of gliding over the total flight time was higher for male *P. helvina* than *C. pireta*, the difference was not significant (Table 2). Finally, male *P. helvina* and *C. pireta* differed in wing-beat frequencies but not in wing-beats over total flight time. In the lab, we did not detect differences between species in female glide durations, proportion of gliding over total flight time, wing-beat frequencies or wing-beats over total flight time (Table 2).

Discussion

Effects of forewing aspect ratio and wing allometry on gliding flight

By reducing drag, a high forewing aspect ratio promotes energy conservation during gliding flight (Dudley 2000, Ancel et al. 2016). Cespedes et al. (2014) showed that Haeterini species in which males glide near the ground to patrol territories (*Pierella*, *Cithaerias*) have a higher forewing aspect ratio than those that lack such behavior (e.g., *Dulcedo*). Here we confirmed that the forewings of our focal species have a high aspect ratio in both sexes, but most importantly, we showed that the higher aspect ratio of male *P. helvina* allows for increased gliding performance (Tables 1 and 2). Intact male *P. helvina* showed longer glide durations than male *C. pireta* in the field and lab, and spent significantly more time gliding during total recorded flight time in the field (Table 2). In contrast, females of focal species had a similar forewing aspect ratio and did not differ with regard to either of these flight parameters. Although female *Pierella* and *Cithaerias* also utilize gliding flight (see Introduction), it is likely that this behavior

is employed more often by patrolling males than by females. Together, our recorded flights in the field and lab appear to support the hypothesis that, in Haeterini, flight behavior can be predicted by forewing aspect ratio alone.

Although male *P. helvina* have a higher forewing aspect ratio than male *C. pireta*, they also possess proportionately larger hind wings (Table 1). Therefore, the aerodynamic gain from a high forewing aspect ratio could potentially be offset by increased drag from an enlarged hind wing in *Pierella*. By filming the same *P. helvina* individuals with intact and experimentally reduced wings, we were able to assess the effect of hind wing area on gliding performance. We showed that hind wing area reduction resulted in both shorter glide durations and reduced proportions of gliding over total flight time, particularly in males (Fig. 5b, Table 3). Thus, our experimental results strongly suggest that gliding performance in *P. helvina* is actually enhanced by their enlarged hind wing area. It has been demonstrated that wing allometry is under genetic control (Frankino et al. 2007), and that independent evolution of butterfly forewings and hind wings can lead to sexual dimorphism in shape (Chazot et al. 2016, Hegedus et al. 2018). By means of comparative analyses, we suggest that male *P. helvina* evolved proportionately larger hind wings than conspecific females as a response to flight demands of their patrolling behavior (Table 1).

Flapping flight performance in *P. helvina* and *C. pireta*

Flapping flight was studied by estimating wing-beat frequencies and the number of wing beats over recorded flight time. Intact male *P. helvina* had higher wing-beat frequencies than male *C. pireta* in the field and lab (Table 2, Fig. 5). The larger thoracic mass of *P. helvina* likely allows higher wing-beat frequencies (e.g., Betts and Wootton 1988), suggesting that male *P. helvina* might be capable of generating the momentum required to sustain longer periods of gliding than *C. pireta*. Although this idea is preliminary and will require further investigation, to our knowledge the balance between flapping and gliding bouts during flight has never been examined for any butterfly species.

Flapping flight in females did not follow the same pattern as in males. In the lab, females of both focal species showed similar wing-beat frequencies and glide durations (Table 2), suggesting that flight pattern (flapping vs. gliding bouts) has a sex-specific component. These

findings are consistent with natural history observations in the field: when butterflies are on the wing, an observer can assess sex-related differences in flight pattern by eye (pers. obs.).

Kinematic analysis of lab-flown individuals of *P. helvina* showed that hind wing reduction decreased escape velocity, and that this effect was strongest in females (Table 3). Insect flight requires the production of both vertical lift and of nose-down torque to avoid stalling at low speeds (Ellington 1999, Dudley 2002). Since increased abdominal mass due to egg loads has been shown to affect flight in female butterflies (Karlsson and Wickman 1990, Almbro and Kullberg 2007, Berwaerts et al. 2002), it is not surprising that experimental reduction of hind wing area diminished escape velocity in females (Table 3). This implies that hind wings play a role in force production during takeoff, and suggests that the increased wing-beat frequency exhibited by females following hind wing area reduction represents a behavioral compensation for diminished lift production during takeoff. Nevertheless, once airborne, relative hind wing area had little or no effect on cruising velocity in these butterflies, suggesting that the role of hind wings in flapping flight may be more important for takeoff than for sustained, undisturbed flapping flight.

P. helvina utilizes ground effect to a greater extent than *C. pireta* in the field

High-speed videography of *P. helvina* and *C. pireta* in the field supported the hypothesis by Cespedes et al. (2014) that these species utilize the drag-reducing properties of ground effect to glide above the rainforest floor. Our work went a step further by assessing natural variation in the range of flight altitudes between species. In the field, *P. helvina* rarely ascends 25cm above the forest floor, while the flight height of *C. pireta* can range to ca. 1.5 m above the ground (pers. obs., see also Alexander and DeVries 2012). These observations suggest that *P. helvina* glides more often within the range of ground effect than *C. pireta*, and may contribute to the observed differences in gliding time during flight between species (Table 2). Finally, using mark-release-recapture, *P. helvina* was estimated to have substantially greater daily dispersal rates than other sympatric Haeterini (Alexander 2014), which could be facilitated by gliding in ground effect.

Concluding remarks

This is the first study to evaluate the aerodynamic role of hind wings in butterflies that regularly employ gliding flight. We demonstrate that although the forewings are reliable predictors of flight behavior in the focal species, hind wing area can have a significant effect on gliding flight performance. Within Haeterini, a tighter association with the forest floor and greater use of ground-effect likely explain the large hind wing area of *Pierella* as compared to *Cithaerias*. We also provide evidence that enlarged hind wings aid in the takeoff flight of female *P. helvina*, and hypothesize that the sex-specific effect of hind wing area reduction is due to larger abdominal mass of females (egg loading). Future work on flight-associated morphology in butterflies should include hind wing area as an additional factor that influences flight performance.

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Supplementary Materials

Supplementary Table S1: Extraction of positional data from two concurrent videos of a male *P. helvina*

Wing-Beat	Frame #	Time (s)	C1 Pixels from Left*	θ1 (rad)	C2 Pixels from Left*	θ2 (rad)	X (m)	Y (m)	C1 Pixels from Top*	θ3 (rad)	Z (m)
Pronation 1	0	0.000	816	0.09	725	0.00	0.145	1.640	509	-0.124	-0.003
Supination 1	17	0.071	824	0.09	734	0.01	0.156	1.655	480	-0.103	0.029
Pronation 2	30	0.125	838	0.10	749	0.02	0.175	1.670	467	-0.094	0.043
Glide	30	0.125	838	0.10	749	0.02	0.175	1.670	467	-0.094	0.043
Supination 2	40	0.167	848.5	0.11	763	0.03	0.195	1.724	460	-0.089	0.047
Pronation 3	47	0.196	850.5	0.11	765.5	0.03	0.198	1.732	459	-0.088	0.048
Glide	47	0.196	850.5	0.11	765.5	0.03	0.198	1.732	459	-0.088	0.048
Supination 3	58	0.242	853	0.12	771	0.04	0.207	1.782	450	-0.082	0.055
Pronation 4	64	0.267	852	0.12	771	0.04	0.208	1.800	446	-0.079	0.059
Glide	64	0.267	852	0.12	771	0.04	0.208	1.800	446	-0.079	0.059
Supination 4	72	0.300	858	0.12	780	0.05	0.223	1.854	440	-0.075	0.062
Pronation 5	79	0.329	860	0.12	785	0.05	0.232	1.911	435	-0.071	0.065
Glide	103	0.429	880	0.14	810.5	0.07	0.277	2.026	431	-0.068	0.063
Supination 5	108	0.450	887	0.14	820	0.08	0.296	2.083	427	-0.065	0.065
Pronation 6	115	0.479	894	0.15	827.5	0.08	0.308	2.094	426	-0.064	0.065

Table S1 represents the first five wing-beats of a single flight of an individual male *P. helvina*, tabulated to illustrate the process of extracting positional data from the confluence of two concurrent videos of a flying butterfly. At each point in the wing-beat cycle, the frame number and distance of a butterfly's head from the left and top of frame (in pixels) were recorded. The head was chosen for position tracking due to its spherical shape and its unchanging position relative to the thorax. Positions were estimated to the nearest half-pixel. While temporal measures were taken for as long as the butterfly remained in view of Camera 1, positional measures were only recorded up to the fifth wing-beat, after which the camera's limited resolution often precluded further analysis. The flight path resulting from this analysis is reported as accurate to the nearest 0.008m, as this was the maximum error recorded during calibration (see Methods). Further details regarding positional triangulation using our stereo camera rig can be found in Supplementary Figure S1.

Supplementary Table S2: Summary of all statistical comparisons with corresponding model assumptions tests.

Forewing Aspect Ratio*					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	t =	p =
P. helvina Males (7) vs. Females (7)	0.356, 0.236	0.125, 0.730	Independent Samples T-Test	3.496	0.002
C. pireta Males (6) vs. Females (4)	0.092, 0.391	0.311, 0.592	Independent Samples T-Test	0.042	0.484
P. helvina Males (7) vs. *C. pireta* Males(6)	0.356, 0.092	0.972, 0.345	Independent Samples T-Test	5.138	1.62E-04
P. helvina Females (7) vs. *C. pireta* Females (4)	0.236, 0.391	3.920, 0.079	Independent Samples T-Test	1.056	0.159
Forewing Area*					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	t =	p =
P. helvina Males (7) vs. Females (7)	0.822, 0.952	0.098, 0.760	Independent Samples T-Test	8.032	1.80E-06
C. pireta Males (6) vs. Females (4)	0.421, 0.878	2.629, 0.144	Independent Samples T-Test	4.187	0.002
P. helvina Males (7) vs. *C. pireta* Males(6)	0.822, 0.421	0.194, 0.668	Independent Samples T-Test	7.957	3.44E-06
P. helvina Females (7) vs. *C. pireta* Females (4)	0.952, 0.878	1.518, 0.249	Independent Samples T-Test	12.02	3.80E-06
Hind Wing Area*					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	t =	p =
P. helvina Males (7) vs. Females (7)	0.503, 0.719	1.845, 0.199	Independent Samples T-Test	4.999	1.55E-04
C. pireta Males (6) vs. Females (4)	0.529, 0.998	1.320, 0.284	Independent Samples T-Test	4.090	0.002
P. helvina Males (7) vs. *C. pireta* Males(6)	0.503, 0.529	1.868, 0.199	Independent Samples T-Test	12.34	4.38E-08
P. helvina Females (7) vs. *C. pireta* Females (4)	0.719, 0.998	1.164, 0.309	Independent Samples T-Test	24.90	6.52E-08
% Hind Wing Area*					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	t =	p =
P. helvina Males (7) vs. Females (7)	0.979, 0.086	0.015, 0.906	Independent Samples T-Test	2.148	0.026
C. pireta Males (6) vs. Females (4)	0.919, 0.765	0.108, 0.751	Independent Samples T-Test	1.790	0.56

P. helvina Males (7) vs. *C. pireta* Males(6)	0.979, 0.919	3.914, 0.073	Independent Samples T-Test	19.34	3.83E-10
P. helvina Females (7) vs. *C. pireta* Females (4)	0.086, 0.765	4.987, 0.052	Independent Samples T-Test	13.08	1.84E-06
Glide Duration					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	H =	p =
P. helvina Males (58) vs. Females (42)	0.098, 1.06E-4	1.193, 0.277	Kruskal-Wallis Test	11.75	6.08E-04
C. pireta Males (61) vs. Females (22)	0.002, 0.226	3.382, 0.070	Kruskal-Wallis Test	4.857	0.028
P. helvina Males (58) vs. *C. pireta* Males (61)	0.098, 0.002	4.475, 0.037	Kruskal-Wallis Test	4.486	0.034
P. helvina Females (42) vs. *C. pireta* Females (22)	1.06E-4, 0.226	12.38, 8.18E-4	Kruskal-Wallis Test	0.941	0.332
P. helvina Wild (124) vs. *C. pireta* Wild (126)	3.28E-8, 1.88E-7	30.68, 7.74E-8	Kruskal-Wallis Test	36.12	1.86E-09
P. helvina Wild (124) vs. Lab (sexes pooled) (100)	3.28E-8, 4.56E-4	8.669, 0.004	Kruskal-Wallis Test	39.99	2.55E-10
C. pireta Wild (126) vs. Lab (sexes pooled) (83)	1.88E-7, 2.31E-4	1.717, 0.192	Kruskal-Wallis Test	12.21	4.75E-04
P. helvina Intact (58) vs. Cut (36) Males	0.098, 0.839	6.253, 0.014	Kruskal-Wallis Test	12.60	3.85E-04
P. helvina Intact (42) vs. Cut (28) Females	1.06E-4, 0.002	0.499, 0.482	Kruskal-Wallis Test	1.221	0.269
Gliding / Total Flight Time					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	U =	p =
P. helvina Males (7) vs. Females (7)	0.044, 0.777	1.237, 0.288	Mann-Whitney U Test	6	0.017
C. pireta Males (6) vs. Females (3)	0.876, 0.543	0.159, 0.702	Mann-Whitney U Test	6	0.548
P. helvina Males (7) vs. *C. pireta* Males (6)	0.876, 0.044	3.438, 0.091	Mann-Whitney U Test	8	0.073
P. helvina Females (7) vs. *C. pireta* Females (3)	0.777, 0.543	1.198, 0.306	Mann-Whitney U Test	12	0.833
P. helvina Wild (16) vs. *C. pireta* Wild (16)	0.853, 0.203	0.097, 0.758	Mann-Whitney U Test	9	3.23E-07
P. helvina Wild (16) vs. Lab (sexes pooled) (14)	0.853, 0.158	1.997, 0.169	Mann-Whitney U Test	1	2.75E-08
C. pireta Wild (16) vs. Lab (sexes pooled) (9)	0.203, 0.853	3.064, 0.093	Mann-Whitney U Test	9	9.50E-05
P. helvina Intact (7) vs. Cut (7) Males	0.044, 0.764	1.534, 0.239	Mann-Whitney U Test	42	0.026

P. helvina Intact (7) vs. Cut (7) Females	0.777, 0.166	0.006, 0.939	Mann-Whitney U Test	38	0.097
Wing-beats / Total Flight Time					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	U =	p =
P. helvina Males (7) vs. Females (7)	0.116, 0.146	1.480, 0.247	Mann-Whitney U Test	31	0.456
C. pireta Males (6) vs. Females (3)	0.042, 0.091	0.799, 0.401	Mann-Whitney U Test	14	0.262
P. helvina Males (7) vs. *C. pireta* Males (6)	0.116, 0.042	1.407, 0.261	Mann-Whitney U Test	24	0.731
P. helvina Females (7) vs. *C. pireta* Females (3)	0.146, 0.091	1.247, 0.297	Mann-Whitney U Test	13	0.667
P. helvina Wild (16) vs. *C. pireta* Wild (16)	0.530, 0.947	0.004, 0.949	Mann-Whitney U Test	234	1.47E-05
P. helvina Wild (16) vs. Lab (sexes pooled) (14)	0.530, 0.065	1.918, 0.194	Mann-Whitney U Test	201	7.57E-06
C. pireta Wild (16) vs. Lab (sexes pooled) (9)	0.947, 0.037	3.457, 0.076	Mann-Whitney U Test	84	0.522
P. helvina Intact (7) vs. Cut (7) Males	0.116, 0.844	0.005, 0.945	Mann-Whitney U Test	10	0.073
P. helvina Intact (7) vs. Cut (7) Females	0.146, 0.127	1.666, 0.221	Mann-Whitney U Test	9	0.053
Wing-beat Frequency					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	H =	p =
P. helvina Males (7) vs. Females (7)	0.291, 0.557	0.794, 0.391	Kruskal-Wallis Test	5.00	0.025
C. pireta Males (6) vs. Females (3)	0.539, 0.287	1.626, 0.243	Kruskal-Wallis Test	1.361	0.243
P. helvina Males (7) vs. *C. pireta* Males (6)	0.219, 0.539	0.015, 0.904	Kruskal-Wallis Test	5.224	0.022
P. helvina Females (7) vs. *C. pireta* Females (3)	0.557, 0.287	1.061, 0.333	Kruskal-Wallis Test	1.052	0.305
P. helvina Wild (16) vs. *C. pireta* Wild (16)	0.106, 0.275	8.402, 0.007	Kruskal-Wallis Test	17.5	2.87E-05
P. helvina Wild (16) vs. Lab (sexes pooled) (14)	0.106, 0.609	4.836, 0.036	Kruskal-Wallis Test	18.33	1.85E-05
C. pireta Wild (16) vs. Lab (sexes pooled) (9)	0.275, 0.144	0.076, 0.785	Kruskal-Wallis Test	7.698	0.006
P. helvina Intact (7) vs. Cut (7) Males	0.219, 0.293	5.128, 0.043	Kruskal-Wallis Test	0.69	0.406
P. helvina Intact (7) vs. Cut (7) Females	0.557, 0.087	0.007, 0.934	Kruskal-Wallis Test	5	0.025

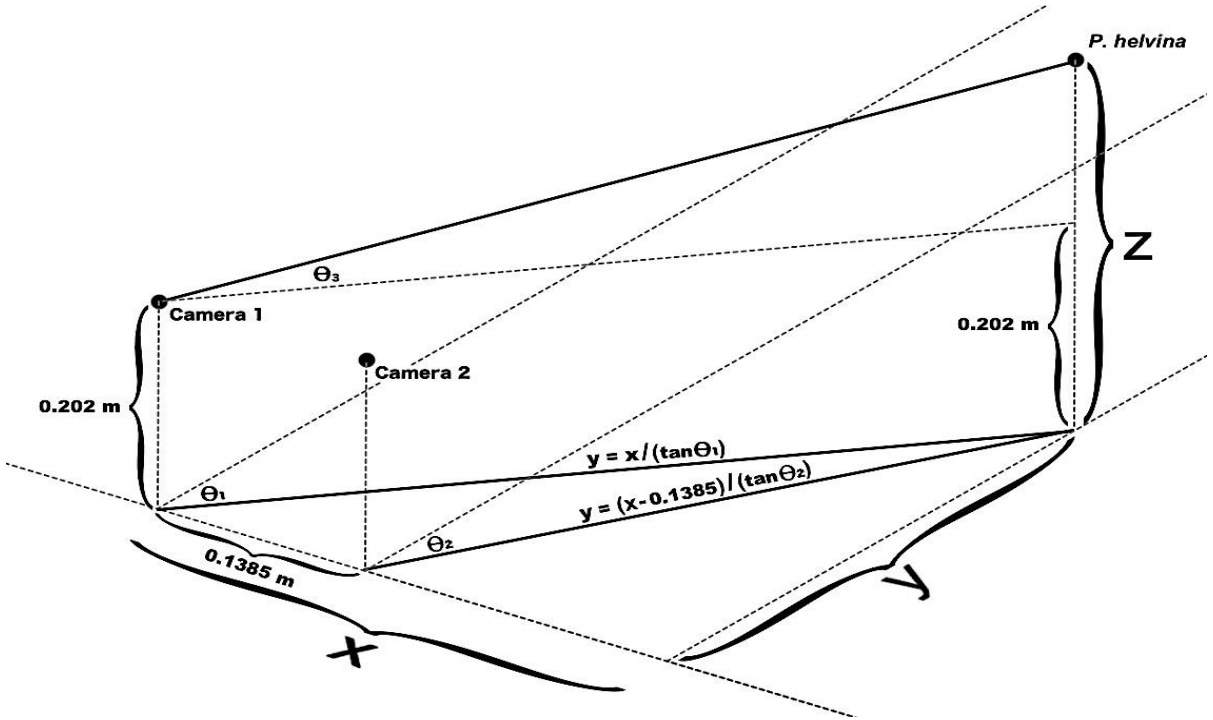
Escape Velocity**					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	U =	p =
P. helvina Intact (12) vs. Cut (13) Males	0.823, 0.907	1.430, 0.244	Mann-Whitney U Test	93	0.437
P. helvina Intact (12) vs. Cut (11) Females	0.949, 0.596	3.383, 0.080	Mann-Whitney U Test	100	0.037
Cruising Velocity**					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	U =	p =
P. helvina Intact (12) vs. Cut (13) Males	0.381, 0.719	3.125, 0.090	Mann-Whitney U Test	91	0.503
P. helvina Intact (12) vs. Cut (11) Females	0.186, 0.260	0.528, 0.476	Mann-Whitney U Test	52	0.413
Sinuosity**					
Samples to be compared	Shapiro-Wilks (p,p)	Levene's Test (F, p)	Comparison Performed	U =	p =
P. helvina Intact (12) vs. Cut (13) Males	5.45E-4, 2.18E-4	0.002, 0.969	Mann-Whitney U Test	23	0.968
P. helvina Intact (12) vs. Cut (11) Females	0.004, 0.002	0.459, 0.505	Mann-Whitney U Test	82	0.347

Table S2 includes the statistical outputs for each set of comparisons performed in this study, with model assumption test results (Shapiro-Wilks and Levene's tests) supporting the use of particular statistical tests for data analysis. Sample sizes (n) are provided in parentheses in the left-hand column, and represent the total butterflies either captured or filmed in the field during a four day period in December, 2016 in the Tirimbina Biological Reserve, Heredia Province, Costa Rica (10◦29'50.3''S; 76◦22'28.9''W). All analyses were performed using the statistical suite JASP for Mac.

* While four undamaged *C. pireta* were captured during this study, only three were capable of flight in the lab, resulting in the sample size disparity between morphological and flight data for female *C. pireta*.

** Although seven undamaged male and female *P. helvina* were capable of flight in the lab, an autofocusing error in our stereo camera rig precluded positional data analysis during some of the flights of two male and two female *P. helvina*, resulting in the sample size disparity between behavioral and kinematic flight data.

Supplementary Figure S1: Diagram of the experimental flight setup depicting the two high-speed video cameras used to film each flight and the associated angles ($\theta_{1\text{-}3}$) used to triangulate a butterfly's position in space.



Positional data analysis was limited to the first five wing-beats of each flight. To extrapolate these positional data from raw videos, it was first necessary to construct a standard curve relating the distance of a butterfly from left-of-frame in pixels to the angle of that butterfly from each camera, measured using ONDE RULERS. These two angles (θ_1 and θ_2), were then used to triangulate the (x,y) position of a butterfly at the beginning (pronation), middle (supination), and end (pronation) of each wing-beat cycle. Height (z) was measured in the same fashion. The construction of a second standard curve was necessary to calculate the vertical angle-from-camera (θ_3) using the distance of a butterfly (in pixels) from top-of-frame. Using this angle, the butterfly height from the ground could be triangulated using the equation:

$$z\ (m) = 0.202 + [\ \tan(\theta_3) * \sqrt{}\ (x^2+y^2)\ ]$$

VITA

The author, born in New Jersey, now resides in New Orleans, LA. He earned his Bachelor's degree in Visual Art from Muhlenberg College in 2012, and joined the University of New Orleans Dept. of Biology graduate program to pursue a Master's degree in 2016, becoming a member of the DeVries/Penz lab shortly thereafter.